J Appl Biotechnol Rep. 2019 Sep;6(3):96-100

doi 10.29252/JABR.06.03.03

Journal of
Applied Biotechnology Reports

Original Article

Investigating the Effect of Fennel and Cinnamon Combined Extract on Spermatogenesis and Testis Tissues in Busulfan Induced Infertile Rats

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Received January 13, 2019; **Accepted** May 21, 2019; **Online Published** September 11, 2019

Abstract

Introduction: Busulfan is one of the common cancer treatment drugs with infertility side effects. Fennel and cinnamon are two medicinal plants with fertility enhancement properties. The aim of this study was to investigate the effects of fennel and cinnamon on busulfan induced infertile rats.

Materials and Methods: Forty male Wistar rats were divided into four groups including: sham group: healthy rats without intervention, control group: Busulfan treated rats, fennel group: busulfan and fennel extract treated, fennel and cinnamon group: busulfan, fennel and cinnamon extract treatment. Testicular tissues were sampled and the testicular physical parameters and spermatogenesis level were evaluated by H & E staining and optical microscopy imaging.

Results: The biggest and the smallest testis lengths were observed in cinnamon + fennel and fennel groups respectively ($P<0.05$). The highest and lowest sperm levels were observed in the cinnamon + fennel group and fennel group respectively ($P<0.001$). The total average of reproductive cells was the most in the cinnamon + fennel group (208.88) and had the least level in the control group (81.2).

Conclusions: The combined extract of fennel and cinnamon significantly protect the testicular tissues against infertility effect of busulfan. However, the fennel extract alone increased the effect of busulfan in rats.

Keywords: Fennel, Cinnamon, Infertility, Spermatogenesis, Testis

Citation: Chegini R, Soleimani P, Sadeghi M, Mohammad Yosef R, Zafari F. Investigating the effect of fennel and cinnamon combined extract on spermatogenesis and testis tissues in busulfan induced infertile rats. J Appl Biotechnol Rep. 2019;6(3):96-100. doi:10.29252/JABR.06.03.03.

Introduction

Spermatogenesis is a complex and multi-stage process. Various factors such as stress, nutrition and certain drugs may affect this process and cause azoospermia or infertility. One of the most common side effects of some chemotherapy drugs is men's azoospermia.[1-3] Busulfan is a leukemia treatment chemotherapy drug which is used before and after bone marrow transplantation. The administration of busulfan in male patients with malignant cancers can lead to permanent or complete infertility.[4,5] It seems that the disorder in spermatogenesis after busulfan treatment is due to the busulfan alkylating effect on DNA, which mainly affects the spermatogonia stem cells.[6,7] So far, various studies have been conducted on the effects of busulfan on rat spermatogenesis during both fetal development[8,9] and maturity stages.[10,11] In all of these studies, azoospermia and testis tissue damaging have been confirmed. Due to the high incidence of infertility in patients undergoing chemotherapy, the use of new therapies has always been considered in different countries. Fennel (*Foeniculum vulgare* Mill) is an indigenous plant of Asia and Iran which is noticeable because of its health benefits in traditional medicine.[12] In recent research, fennel has been considered due to its antioxidant and anti-inflammatory properties.[13,14] Also, a group of flavonoids called phytoestrogen exist in fennel seeds that have a competitive effect on binding to estrogen receptors. Actually, phytoestrogen maintains the balance of unwanted reactions resulted from the changes in feminine hormones before and after menopause in women.[15] However, no studies have yet been done on the effects of fennel in spermatogenesis and infertility.[16] Cinnamon (*Cinnamomum zeylanicum*) is a widely used alternative medicine that is indigenous to India. It is an aromatic herbal plant that contains many antioxidant compounds such as cinnamon aldehyde, terpenes, cinnamyl alcohol and safrole.[17] Cinnamon is an extremely important plant in traditional medicine and many properties, including fat reduction, antibacterial properties, antifungal properties and sexual enhancement properties have been reported to it.[18,19] The

results from various studies confirm the fact that cinnamon extract has been effective in improving the function of the reproductive system and increasing spermatogenesis in rats.[20] Considering the increasing infertility in the males population and on the other hand, little research on the effect of fennel and cinnamon in the male reproductive system, in this study the synergistic effect of fennel and cinnamon extract and also the effect of fennel alone on the reproductive system of busulfan induced infertile rats has been investigated.

Materials and Methods

Animal Preparation and Infertility Model

For this study, 40 adult male Wistar rats (180-200 g) were prepared from the Razi Institute Center of Karaj, Iran. The animals were administrated under standard laboratory conditions according to the standard protocol at Qazvin University of Medical Sciences Animal Breeding and Development Center. The oligospermic model was performed by intravascular busulfan injection in two different dosages: the first dosage of 25 mg/kg of busulfan and the second dosage of 15 mg/kg were administered 14 days later.

Animals Grouping and Treatment

The rats were divided into 4 groups including sham group: healthy rats without any intervention, control group: rats treated with busulfan in two doses of 25 mg/kg and 15 mg/kg with a 14 day interval, fennel group: Busulfan treated rats following with a fennel extract (100 mg/kg) intraperitoneal injection for a period of 5 days, and the fennel+cinnamon group: busulfan treated rats with a fennel (100 mg/kg) and cinnamon (200 mg/kg) extract intraperitoneal injection for a period of 5 days.

Preparation of Fennel and Cinnamon Extract

Plants extraction was done by a pharmacologist at the Iranian Research Institute of Pharmaceutical Sciences according to the following protocol: 10 g of plant powder was added to 1 L of ethanol and extracted for 2 hours by ultrasonic apparatus. The extract was then placed in dark under laboratory conditions (25°C) for 24 hours. After filtration, the extract was concentrated by rotary evaporation (vacuum distillation) at a temperature below 50°C. The extracts and fractions were stored at -18°C until they were consumed.

Sampling and Assessments

According to the spermatogenesis period of rats which is 4 weeks,[21] the animal's testis samples were prepared after four weeks from the beginning of the treatment. For this purpose, after the anesthetic, the testis were removed and the parameters of weight, length and width of them were measured and the specimens were then placed within a 48 hour Buffin fixture. Then, the specimens were transferred to the microprocessor. After being overrun, the slices were stained with H & E staining. The ImageJ software was used for the slides imaging.

Statistical Analysis

The results of the study were analyzed by SPSS and ANOVA. The *t* test was used to evaluate the significant difference between groups and $P \leq 0.05$ was considered as a significant level for all calculations.

Results

Spermatogenesis Level of Studied Groups

Four weeks after treatments, the most spermatogonia number was observed in the cinnamon + fennel group (73.19 ± 11.30) and the lowest was observed in the fennel group (49.32 ± 13.53). The difference between these two groups was significant ($P < 0.05$). The highest and lowest spermatocyte numbers were observed in cinnamon + fennel (62.50 ± 13.70) and control group (13.67 ± 5.27) respectively. The highest and lowest spermatids numbers were observed in the cinnamon + fennel (73.19 ± 11.30) and control groups (15.7 ± 38.67) respectively. Generally, the highest and lowest number of reproductive cells (spermatogonia, primary spermatocyte and spermatids) belonged to the fennel + cinnamon (208.88) and control groups (81.2) (Table 1 and Figure 1).

Testis Weight of the Studied Groups

In evaluation of the testis weight in the studied groups, the testis weight in the fennel + cinnamon group was significantly higher than the other groups. Among the groups, the highest testis weight (1.66 ± 0.17 g) belonged to the fennel + cinnamon group and the lowest testis weight (1.22 ± 0.34 g) belonged to the fennel group, with a significant difference between the two groups ($P < 0.05$). The weight of the testis in the fennel group was even lower than the busulfan group (1.25± 0.89 g), which indicates the negative effect of fennel on the testicular (Table 2).

Testis Length and Width in Studied Groups

In the evaluation of the size and length of the testis in the studied groups, the highest and lowest testis lengths was observed in fennel + cinnamon (2.32 ± 0.26 cm) and fennel (1.56 ± 0.12 cm) groups respectively, and the difference between the two groups was statistically significant ($P < 0.001$). The combined extract group had the best results regarding the size of the testis compared to the control group. The testis length in the fennel group (1.56 ± 0.12) was even lower than the busulfan treated group (2.17 ± 0.18). In the evaluation

Table 1. Reproductive Cells Count in the Study Groups on the 14th Day

	Spermatogonia	Primary Spermatid	Spermatid	*P* Value
Sham	69.17 ± 12.28	76.50 ± 16.67	85 ± 13.9	-
Control	51.83 ± 23.37	13.67 ± 5.27	15.7 ± 38.67	-
Fennel	49.32 ± 13.53	57.20 ± 12.95	65.02 ± 18.8	-
Fennel + Cinnamon	73.19 ± 11.30	62.50 ± 13.70	73.19 ± 11.30	0.001

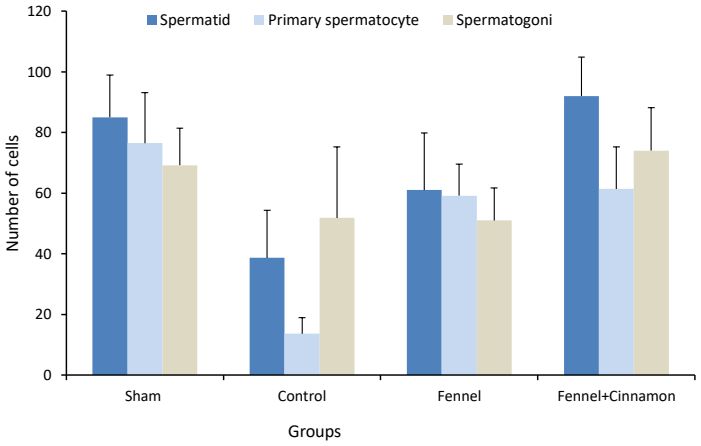

Figure 1. Comparison of Reproductive Cells in the Studied Groups. The average number of spermatogonia cells in the fennel + cinnamon group was increased, but this increase was significant in comparison with the fennel group (P <0.05). The number of primary spermatocytes in the control group (busulfan) was significantly lower than the other groups (P<0.001). The number of spermatids in the combined group increased compared to the other groups, which was significantly higher than the fennel group and control Was there (P<0.001).

of the testis width, the biggest and smallest testis width were observed in the fennel+cinnamon (1.41± 0.48 cm) and fennel (1.11± 0.16 cm) groups respectively (Figure 2 and Table 2).

Discussion

In this study, the effect of fennel and cinnamon extract was investigated on busulfan-induced infertile rats. According to the findings of the present study, fennel and cinnamon mixed extracts repair the testis tissue and significantly increase the spermatogenesis level in busulfan-induced infertile rats.

Table 2. Testicular Physical Parameters of the Studied Groups on the 14th Day

	Weigh (g)	Length (cm)	Width (cm)
Sham	1.47±0.16	2.50±0.25	1.48±0.38
Control	1.25±0.89	2.17±0.18	1.36±0.19
Fennel	1.22±0.34	1.56±0.12	1.11±0.16
Fennel + Cinnamon	1.66±0.17	2.32± 0.26	1.41±0.48

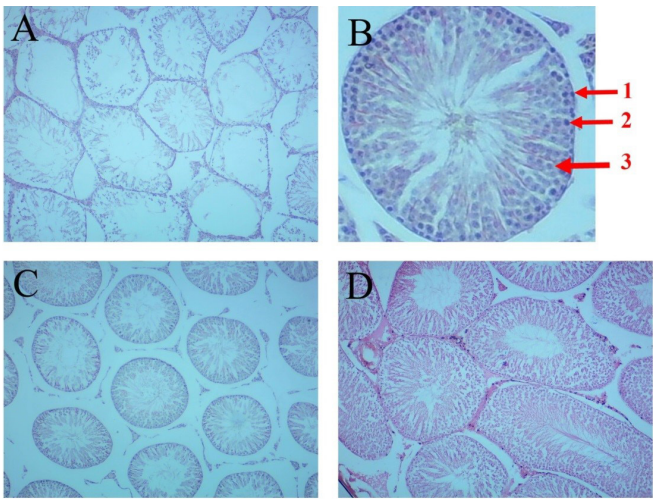

Figure 2. H & E Staining Image of Seminal Tube Sections of the Studied Groups. A: Control group (Busulfan), B: Sham group (B1: spermatogonia, B2: primary spermatocyte, B3: spermatid), C: Fennel group, D: Fennel + Cinnamon group.

This is while the use of fennel extract alone exacerbated the side effects of busulfan and led to the loss of testis tissue and reduction of spermatogenesis.

According to the results in new research, cinnamon could increase sperm quality parameters such as population, viability and motility. Herbal antioxidants eliminate and suppress reactive oxygen species (ROS) formation, and the reduction of ROS is a crucial factor in the production of sperm cells and optimization of the fertility rate.[22,23] More research seems to be necessary to confirm the pharmacological and toxicological effects of this medical herb on body tissues.[24]

Antol is a substance that acts like estrogen. It has been reported that its consumption in men decreases testosterone levels and depletes sexual power.[25] These observations are consistent with the findings of Mansouri et al who have reported that fennel leads to a reduction of blood testosterone and spermatogenesis in rats.[26] In a study on the effect of intraperitoneal injection of fennel extract on rat spermatogenesis, Rezaei Ahvanouei et al reported that the fennel extract significantly reduced the number of spermatid.[27] In the present study, the most reduction in testis size was observed in the simultaneous use of fennel and busulfan, which confirms previous findings.

The study of the effects of the aqueous extract of fennel seed by Hosseini Ahar et al indicated that busulfan injection reduced testis weight and increased serum levels of MDA.[28] Ghasemi et al reported that busulfan reduced the production of spermatogonia cells in mice.[29] This finding suggest that fennel exacerbates the infertility effects of busulfan. Antol and fennel are to important compounds present in the fennel extract.[30,31] Contrary to the results of this study, Mansouri and colleagues reported that histological and morphometrical studies of testis tissue indicated a reduction in sperm cell count in the experimental groups compared to the control group. They suggested that hydroalcoholic extract of fennel seed had inhibitory effect on spermatogenesis and fertility.[26] One of the main reasons for reduction in the cell count could be due to the existing compounds in the herb extract that inhibited cell division, particularly flavonoids. According to the findings of

recent studies, flavonoids could restrain DNA replication and inhibit cell division, as well as stimulate apoptosis by affecting cell DNA.[32]

Garawani et al reported that the consumption of fennel oil in etoposide defective rats caused reduction of sperm defects and increased mature sperm production, which was due to the anti-oxidant properties of the fennel extract.[33] The findings of Garawani et al's study about the effect of fennel on spermatogenesis are contradictory to the findings of this study. In evaluation of fennel and cinnamon combined extract, a significant increase has been observed in the testis reproductive cells especially in the spermatogonia cells. In related studies, Khaki et al reported that consuming cinnamon alone or mixed with fennel will significantly increase the number, motility and survival of sperms.[34]

Conclusions

In conclusion, the findings of this study suggest that the hydro-alcoholic extract of fennel and cinnamon could repair the damaged testis tissues and improve the spermatogenesis process in busulfan induced rats and it seems that these plant compounds can be used to treat chemotherapy-induced infertility in the future.

Authors' Contributions

FZ and RC designed the study and performed the experiments. MS analyzed the data and wrote the paper.PS and RY performed experiments and collected samples. FZ analyzed the data and critically reviewed the manuscript.

Conflict of Interest Disclosures

The authors declare they have no conflicts of interest.

Funding

This project was funded by the financial support of the Student Research Committee, School of Medicine, Qazvin University of Medical Sciences.

Acknowledgments

The authors would like to sincerely thank the Student Research Committee of Qazvin University of Medical sciences for providing the needed facilities of this study.

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